Chemical Mixture

No CAS #
Swiss CD-1 mice, at 10, 50, and 100X
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This mixture* (MIX), a cocktail of 25 organic compounds and metals found in contaminated groundwater supplies, was tested as part of a larger program evaluating the possible effects of this mixture on a number of organ systems (Yang et al., Fundam Appl Toxicol 13:366–376 [1989]). This study evaluated Swiss CD-1 mice by the RACB protocol (Heindel et al., Fundam Appl Toxicol 25:9–19 [1995]). Doses of 10, 50, and 100× in water were selected, based on previous National Toxicology Program data. (A standard solution, designated "1000×", contained the greatest possible concentration of all 25 chemicals in the mix. The dose levels represent 0, 1, 5, and 10% of this "1000×" level, or "0, 10, 50, and 100× levels"). The concentrations of individual components are specified in Figure 2 of the final technical report.

For the first generation (Task 2), water consumption was decreased, although feed consumption and body weight were unchanged. Generally, the high dose mice were exposed to 6 to 9 times more of the test agents than were the low dose mice. Lethargy and piloerection were noted during Task 2, but these were not dose related.

There was a monotonic, small decrease in the number of female pups produced in each litter, noted for both the middle and high dose groups. The mean number of live females per litter was (from control to high dose, resepctively) 6.9, 7.0, 6.2*, and 5.7* (* indicates significantly different from controls). This reduced the number of live pups per litter by 10% at the high dose, and significantly changed the sex ratio for these two groups. No other reproductive nor general changes were detected.

The last litter from each dose level was reared until weaning. Exposure to this mixture had no adverse effect on body weights or mortality prior to weaning.

Task 3 (the crossover study) was not conducted since fertility and reproductive effects in Task 2 were minimal. Thus, the Task 2 control and high dose adults were subject to a limited necropsy: body weights were not different between control and treated males or females. Female estrous cycle length was the same for both groups, while testis weight and spermatid count was also not different between controls and treateds.

Because of the importance of this exposure, all groups were raised for the mating of the second generation. Again, the number of live female pups per litter was decreased at the high dose (by 19%), though total number of live pups was unchanged. Adjusted live F_2 pup weight was reduced by 9% in the high dose group. No other differences were noted across the dose levels, despite reduced fluid intake at the middle and high dose levels of approximately 20 and 27%, respectively.

After the F_2 pups were evaluated and removed, the F_1 adults from all dose levels were killed and necropsied. While male body weights were unaffected by exposure to this mixture, adjusted kidney weights were increased at the middle and high dose levels by 8 and 14%, respectively, while adjusted seminal vesicles weight was increased by 18, 12, and 13%, respectively, in the low to high dose groups. Epididymal sperm count (number per milligram cauda) was reduced by 16 and 20% at the low and the high dose groups, respectively, while the 10% reduction at the middle dose was not significant. In parallel with the reduced epididymal count, there was a 20% reduction in the number of spermatids per milligram testis at the high dose. For females, body weight was unchanged, and adjusted kidney weight was increased by 11 and 14% in the middle and high dose groups. Antemortem estrous cyclicity was unchanged by any level of MIX consumption. Both males and females at the high dose only showed an increase in microscopic nephropathy, the only microscopic lesion observed.

In conclusion, this mixture caused a significant change in the sex ratio in mice in both generations, and reduced adjusted pup weight for the F_2 litters. In the second generation, this occurred only at and above doses that caused significant increases in kidney weight and microscopic pathology. It is not possible to attribute the change in sex ratio to a specific gender, a change that is unique among the RACB studies performed to date. The lack of structural male toxicity replicates another study which focused solely on male reproductive toxicity in B6C3F1 mice (Chapin et al., Fundam Appl Toxicol 13:388–398 [1989]).

*Chemical Mixture (concentrations in ppm for 100× group): acetone (53), Arochlor 1260 (0.01), arsenic (9), benzene (12.5), cadmium (51), carbon tetrachloride (0.4), chlorobenzene (0.1), chloroform (7), chromium (36), di(2-ethylhexyl)phthalate (0.015), 1,1-dichloroethane (1.4), 1,2-dichloroethane (40), 1,1-dichloroethylene (0.5), 1,2-trans-dichloroethylene (2.5), ethylbenzene (0.3), lead (70), mercury (0.5), methylene chloride (37.5), nickel (6.8), phenol (29), tetrachloroethylene (3.4), toluene (7), 1,1,1-trichloroethane (2), trichloroethylene (6.5), xylenes (1.6).

Summary: NTP Reproductive Assessment by Continuous Breeding Study.

NTIS#: **91158444**
Chemical: **Chemical Mixture**
CAS#: **None**
Mode of exposure: **Water**
Species/strain: **Swiss CD-1 mice**

F_0 generation — Dose concentration →	10X	50X	100X
General toxicity	Male, female	Male, female	Male, female
Body weight	—, —	—, —	—, —
Kidney weight[a]	•, •	•, •	•, •
Liver weight[a]	•, •	•, •	•, •
Mortality	—, —	—, —	—, —
Feed consumption	—, —	—, —	—, —
Water consumption	—, —	—, —	↓, ↓
Clinical signs	—, —	—, —	—, —
Reproductive toxicity			
x̄ litters/pair	—	—	—
# live pups/litter; pup wt./litter	—, —	—, —	↓, —
Cumulative days to litter	—	—	—
Absolute testis, epididymis weight[a]	•, •	•, •	—, •
Sex accessory gland weight[a] (prostate, seminal vesicle)	•, •	•, •	•, •
Epidid. sperm parameters (#, motility, morphology)	•, •, •	•, •, •	•, •, •
Estrous cycle length	•	•	—

Determination of affected sex (crossover)	Male	Female	Both
Dose level	•	•	•

F_1 generation — Dose concentration →	10X	50X	100X
General toxicity	Male, female	Male, female	Male, female
Pup growth to weaning	—, —	—, —	—, —
Mortality	—, —	↑, —	—, ↓
Adult body weight	—, —	—, —	—, —
Kidney weight[a]	—, —	↑, ↑	↑, ↑
Liver weight[a]	—, —	—, —	—, —
Feed consumption	—, —	—, —	—, —
Water consumption	—, —	↓, ↓	↓, ↓
Clinical signs	—, —	—, —	—, —
Reproductive toxicity			
Fertility index	—	—	—
# live pups/litter; pup wt./litter	—, —	—, —	—, ↓
Absolute testis, epididymis weight[a]	—, —	—, —	—, —
Sex accessory gland weight[a] (prostate, seminal vesicle)	—, ↑	—, ↑	—, ↑
Epidid. sperm parameters (#, motility, morphology)	↓, —, —	—, —, —	↓, —, —
Estrous cycle length	—	—	—

Summary information	
Affected sex?	Unclear
Study confounders:	None
NOAEL reproductive toxicity:	50X
NOAEL general toxicity:	10X
F_1 more sensitive than F_0?	No
Postnatal toxicity:	No

Legend: —, no change; •, no observation; ↑ or ↓, statistically significant change ($p<0.05$); —, —, no change in males or females. [a]Adjusted for body weight.